

Developmental mortality increases sex-ratio bias of a size-dimorphic bark beetle

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Abstract. 1. Given sexual size dimorphism, differential mortality owing to body size can lead to sex-biased mortality, proximately biasing sex ratios. This mechanism may apply to mountain pine beetles, *Dendroctonus ponderosae* Hopkins, which typically have female-biased adult populations (2 : 1) with females larger than males. Smaller males could be more susceptible to stresses than larger females as developing beetles overwinter and populations experience high mortality.

2. Survival of naturally-established mountain pine beetles during the juvenile stage and the resulting adult sex ratios and body sizes (volume) were studied. Three treatments were applied to vary survival in logs cut from trees containing broods of mountain pine beetles. Logs were removed from the forest either in early winter, or in spring after overwintering below snow or after overwintering above snow. Upon removal, logs were placed at room temperature to allow beetles to complete development under similar conditions.

3. Compared with beetles from logs removed in early winter, mortality was higher and the sex ratio was more female-biased in overwintering logs. The bias increased with overwinter mortality. However, sex ratios were female-biased even in early winter, so additional mechanisms, other than overwintering mortality, contributed to the sex-ratio bias. Body volume varied little relative to sex-biased mortality, suggesting other size-independent causes of male-biased mortality.

4. Overwintering mortality is considered a major determinant of mountain pine beetle population dynamics. The disproportionate survival of females, who initiate colonisation of live pine trees, may affect population dynamics in ways that have not been previously considered.

Key words. Body size, *Dendroctonus ponderosae*, developmental mortality, mountain pine beetle, Scolytinae, sex ratio, sex-biased mortality, sexual size dimorphism, size-biased mortality.

Introduction

One of the fundamental life history traits is offspring sex ratio. If daughters and sons are equally costly, the basic expectation for most organisms is that the sex ratio should be equal (Fisher, 1930). Deviations from equal sex ratio therefore demand explanation. However, if the measured sex ratio is not the primary (fertilised egg) sex ratio, as is often the case, skewed sex ratios may arise if there is sex-biased mortality during development. One cause of sex-biased developmental mortality may be sexual size dimorphism. Body size can affect mortality particularly in response to stressors (Bortolotti, 1986; Kalmbach

et al., 2005) such as desiccation (Terblanche *et al.*, 2005), heat stress (Reim *et al.*, 2006), energetic demands (Sheldon *et al.*, 1998; Vedder *et al.*, 2005) and ability to attain resources (Anderson *et al.*, 1993; Oddie, 2000). Consequently, sexual size dimorphism may determine differential susceptibility (Bosch, 2008), leading to sex-biased mortality (Oddie, 2000; Lee *et al.*, 2010). The direction of the bias, towards the larger or smaller sex, may vary. For example, when resources are scarce, the larger sex may experience higher mortality as a result of greater energy demands (Vedder *et al.*, 2005). Alternatively, the smaller sex may experience higher mortality when siblings compete for resources (Anderson *et al.*, 1993; Oddie, 2000). The relationship between sexual size dimorphism and sex ratio has been extensively examined in birds (reviewed in Benito & Gonzalez-Solis, 2007; Kalmbach & Benito, 2007; Jones

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et al., 2009), but the generality of these studies for other taxa is unclear.

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Curculionidae: Scolytinae), consistently has female-biased population sex ratios (approximately 2:1) for emergent adults (Reid, 1958; McGhehey, 1969; Safranyik, 1976; Amman, 1984; Amman & Bartos, 1991). The bias is usually attributed to male-biased mortality in the juvenile stage (Watson, 1971; Safranyik, 1976), but this hypothesis has not been formally tested. Mountain pine beetles typically colonise the bole of live lodgepole pine, *Pinus contorta* var. *latifolia* Englem, to reproduce in the phloem (Raffa & Berryman, 1983). Females initiate individual breeding sites (attacks) on trees, and as a result of active defence by trees, mass attack is needed to overwhelm the defences and kill the tree to allow successful reproduction. If successful, females mate and lay eggs in vertical galleries during late summer and offspring typically overwinter as larvae or pupae within the inner bark, resuming development during spring and emerging as adults the following summer (Safranyik & Carroll, 2006). Mountain pine beetles have female-biased sexual size dimorphism; adult females are 13% larger (pronotum width) than males (McCambridge, 1974; Safranyik, 1976), which could leave males more vulnerable to certain stresses. Juvenile mortality can result from defences produced by the host tree, desiccation, intra- or inter-specific competition, parasites, pathogens or predators (Cole, 1973; Amman, 1984; Rankin & Borden, 1991). The higher surface area to volume ratio of the smaller males may result in greater desiccation stress. However, freezing is considered to be the major cause of death in overwintering mountain pine beetles (Cole, 1981; Safranyik & Carroll, 2006), which we propose may vary depending on height of the breeding gallery on the tree bole and the insulating effect of snow. The effect of snow on the survival of overwintering mountain pine beetles has not been rigorously tested and may have important implications for population growth. We expect higher survival of overwintering juveniles below snow than above snow (Lombardero *et al.*, 2000).

In this study, we test the effects of overwintering mortality on the sex ratio and body size of emerging mountain pine beetles. We hypothesise that disproportionate mortality of males during development causes the typical female bias in adult populations of mountain pine beetles. If mortality is size dependent, survival rate should be positively related to size for each sex, with males experiencing proportionally higher mortality, as they are smaller on average and are likely more susceptible to the stress of overwintering temperatures and desiccation. Such size-dependent mortality occurs among adult mountain pine beetles (Safranyik, 1976) and is tested here for juveniles.

Materials and methods

Study population

This study was conducted near Canmore, Alberta (50.94°N, 115.32°W, elevation 1719 m) over the winter of 2008–2009. We used 15 lodgepole pine trees that had been naturally colonised by mountain pine beetles during the summer of 2008. In our study population, females were 11% larger

than males based on pronotum width (mean ± SE: females: 2.02 ± 0.004 mm, $n = 2085$, males: 1.82 ± 0.004 mm, $n = 1120$) and length (females: 4.95 ± 0.01 mm; males: 4.46 ± 0.01 mm), and 37% larger based on volume (described below; females: 10.76 ± 0.06 mm³; males: 7.85 ± 0.05 mm³) (all $P < 0.0001$).

Study design

To assess the relationships between mortality, sex ratio and body size, two logs from each tree were subjected to one of three treatments differing in their exposure to severe winter temperatures. The study trees were felled on 4 December 2008, by which time reproduction has ceased and the initial sex ratio was set. At this time, beetles were presumably cold hardened through the accumulation of cryoprotectants such as glycerol (Sømme, 1964) but likely had not experienced temperatures cold enough to cause significant mortality (0 h < -20 °C: Fig. 1; mortality can be expected at temperatures below -20 °C for larvae in early winter: Logan *et al.*, 1995; Bentz & Mullins, 1999). The average tree diameter (± SE) of the 15 sample trees was 23.1 ± 0.1 cm, with a range from 17.1 to 30.7 cm. From each tree, a 2-m section of the trunk was removed starting approximately 50 cm from the base, and cut into six 30-cm logs with a central 15-cm log. Logs were selected from this portion of the trunk because it typically has the highest density of attacks (Safranyik *et al.*, 1974). The density of brood initiated (termed attack density) at 1.5 m from the base of a tree ranged from 10 to 285 attacks/m², with an average of 97.1 ± 16.8 attacks/m² ($N = 15$ trees). The range of maternal gallery density in logs was 0 to 40.31 m m⁻², with an average (± SE) of 21.56 ± 2.82 m m⁻² ($N = 15$ trees). Because the density of mountain pine beetle attacks, number of emerging beetles and beetle body size could vary with height of attack even within this 2-m section (Safranyik & Jahren, 1970), one

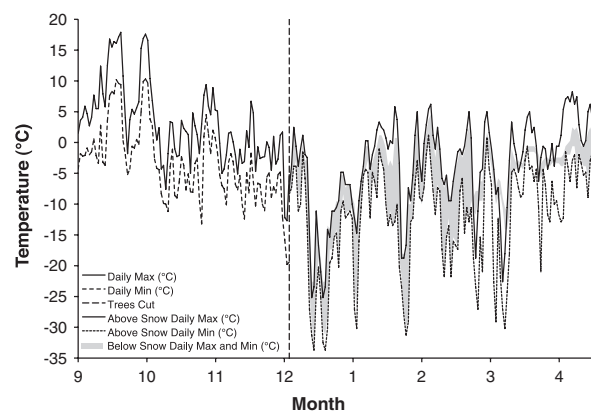


Fig. 1. Daily maximum and minimum temperatures (°C) from 1 September 2008 to 18 April 2009. Study trees were cut 4 December 2008. Data prior to 4 December 2008 were from the nearest Environment Canada weather station, 9 km away (Climate Identifier 305MGFF). Data after 4 December 2008 were from the portable weather station that recorded temperatures above and below snow at the study site. Temperatures from the two weather stations were highly correlated ($r > 0.8$) with the study site approximately 1 °C warmer.

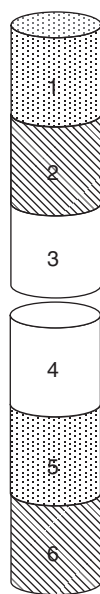


Fig. 2. For each tree, one upper and one lower log were randomly assigned to each of the three treatments. In this example, logs 3 and 4 were assigned to the pre-winter treatment, 1 and 5 to the below snow treatment, and 2 and 6 to the above snow treatment.

lower and one upper log from each tree was randomly assigned to each of the three treatments (i.e. two logs per treatment per tree, Fig. 2). We sealed the ends of all logs with pruning spray to reduce drying and desiccation stress on the beetles (Adams & Six, 2007).

The three treatment groups were termed pre-winter, below-snow and above-snow treatments. For the pre-winter treatment, on the day of felling, logs were moved to the laboratory for completion of development at room temperature (21 °C). Logs in the below-snow treatment were left to overwinter standing on the forest floor in their source stand where they became insulated by snow. As north phloem temperatures tend to be more similar to air temperatures (Bolstad *et al.*, 1997), logs in the above-snow treatment were strapped to the north sides of standing trees approximately 1.5 m above ground level and overwintered in their source stand. No evidence of woodpecker predation was observed on exposed logs. Air temperature and relative humidity (RH) above and below snow were recorded by a HOBO weather station data logger (model: H21-001). Below- and above-snow logs were removed from the forest to the laboratory on 18 April 2009, to allow for completion of development and collection of surviving beetles. In all three treatments, offspring began to emerge within 2 weeks of being placed at room temperature and emergence continued over the next 4 months.

Diameter at breast height (approximately 1.5 m height), phloem thickness, and beetle development stage were measured from a central 15-cm log at the time that treatments were applied to each tree. A dissecting microscope with an ocular micrometer (12.5× magnification) was used to measure phloem thickness from the average of three equally spaced locations on the circumference of the log. The bark was peeled to expose

the beetle larvae so that we could assess whether they were predominantly early instar (first or second), or late instar (third or fourth), based on relative head capsule size.

To collect emerging beetles, logs were individually enclosed in cages made from cylindrical cardboard concrete-forms with the ends tightly covered with circular aluminium pans. Pin holes in the aluminium allowed for air circulation within these enclosures. The cages were dark except for a 2.5-cm hole near the bottom, which led to a transparent collection jar. Beetles were attracted to the light from this hole and emerged into the collection jars, which were emptied every 2–4 days. Emerged individuals were counted and stored in the freezer (–15 °C), thus killing them. A dissecting microscope with an ocular micrometer was used to measure the body length (anterior end of head to posterior end of elytra, 16× magnification) and pronotal width (40× magnification) of each beetle. Sex was determined by examining the seventh tergite for a stridulation organ that only males bear (Lyon, 1958). When emergence ceased after approximately 4 months, the diameter and length of each log were measured to calculate its surface area. The bark was removed to measure the total length of all maternal galleries along which eggs were laid.

Statistical analyses

To determine the effect of overwintering treatments on offspring production, sex ratios and body size, we used general and generalised linear mixed (GLM) models (SAS 9.2; SAS Institute Inc., Cary, North Carolina). These analyses accounted for the repeated-measure design with two fully crossed within-subject fixed factors (treatment and log height) and tree and log (nested within tree) as random factors. These analyses also considered, for each log, the total length of maternal galleries (log_e-transformed, m), surface area (m²) and mean phloem thickness (mm) as covariates. Separate analyses replaced treatment with a mortality index calculated based on expected beetle offspring production (see Results) as a predictor of sex ratio and body size. The analysis of body size also included sex and its interactions with treatment and log height. The Kenward–Roger method was used to account for the repeated-measure design resulting in fractional degrees of freedom (Kenward & Roger, 1997).

Response variables with non-normal error distributions (offspring production and sex ratio) were analysed with GLM models (PROC GLIMMIX). The most appropriate error distribution was selected based on deviance and the variance-covariance matrix was subsequently selected based on the lowest QIC value (PROC GENMOD). The analysis of offspring production (the number of offspring that completed development and emerged as adults) applied a negative binomial distribution with the log link function and a Toeplitz variance-covariance matrix. Analysis of the proportion of female offspring per log applied a binomial distribution with the logit link function and a heterogeneous compound-symmetry variance-covariance matrix.

Effects on body volume were assessed with a general linear mixed model (PROC GENMOD). We estimated body volume

Table 1. Number of hours and days with at least one value below -30 , -25 and -20 °C and average \pm (SE) hourly temperature and relative humidity (RH) for overwintering treatments recorded by a portable weather station.

Time		N	Below snow		Above snow		χ^2	P
			Count	Count	Count	Count		
Number of hours	< -20 °C	3238	239	376	33.72	<0.0001		
	< -25 °C	3238	115	203	25.61	<0.0001		
	< -30 °C	3238	28	58	10.61	0.0011		
Number of days	< -20 °C	136	16	29	4.50	0.0339		
	< -25 °C	136	9	16	2.16	0.1418		
	< -30 °C	136	4	8	1.39	0.2376		
		<i>n</i>	Mean \pm SE	Mean \pm SE	<i>t</i> *	P		
Temperature (°C)		3238	-7.17 ± 0.12	-7.98 ± 0.15	13.11	<0.0001		
RH (%)		3238	82.79 ± 0.20	68.14 ± 0.26	55.82	<0.0001		

*Paired *t*-test assuming equal variance.

Bold font indicates significance ($\alpha < 0.05$).

as an ellipsoid with length as the major axis and pronotal width as the minor axes, and used the square root of volume in analyses. This variable was normally distributed with homogeneous variance. All means are presented with standard errors, unless otherwise indicated.

Results

The applied treatments were successful at providing differences in overwintering environment for the developing mountain pine beetles. As expected, average outdoor temperature above the snow was colder, by 11%, than below the snow and the RH was lower, by 15% (Table 1). Compared with logs that became submerged in snow ($n = 30$), the logs in the above-snow treatment ($n = 30$) were exposed 53% longer (hours) and 81% more frequently (days) to temperatures less than -20 °C (Table 1). Temperatures ranged from -33.7 to 8.2 °C above snow and -32.5 to 4.6 °C below snow (Fig. 1). The mean minimum above snow was -12.3 ± 0.7 °C and -9.8 ± 0.7 °C below snow. The mean maximum above snow was -3.8 ± 0.6 and -4.6 ± 0.5 °C below snow.

Daily minimum and maximum temperatures were less variable below snow than above snow (Fig. 1).

Beetle production

Prior to overwintering, all trees ($N = 15$) contained juvenile mountain pine beetles, which were predominantly early instar larvae. Two trees failed to produce any adult beetles, regardless of treatment. The number of beetles produced per log differed significantly among treatments after controlling for total length of maternal galleries surface area and phloem thickness (Table 2). The number of beetles produced increased with the total length of maternal galleries (Table 2, partial regression coefficient \pm standard error: 1.38 ± 0.34). Relative to pre-winter logs, logs that overwintered produced less than half as many beetles (Fig. 3a, $F_{1,21,23} = 77.15$, $P < 0.0001$; below snow: 64.4% fewer beetles, above snow: 98.4% fewer beetles). Above-snow logs produced 95.6% fewer beetles than did below-snow logs (Fig. 3a; $F_{1,26,17} = 60.11$, $P < 0.0001$). Other measures of beetle productivity, beetles per metre of maternal

Table 2. Tests of fixed effects on (a) offspring production (number of individuals per log), (b) sex ratio, and (c) body volume (mm^3) as a function of treatment (pre-winter, below-snow, and above-snow) and other covariates.

Model Effects	(a) Offspring production			(b) Proportion female			(c) Square root body volume		
	d.f.	F	P	d.f.	F	P	d.f.	F	P
Treatment	2,25.74	41.41	<0.0001	2,18.67	6.45	0.0074	2,60.3	0.11	0.8978
Log height	1,41.09	3.79	0.0583	1,12.02	2.38	0.1492	1,104	0.01	0.9345
Treatment \times Log height	2,27.61	0.27	0.7619	2,18.42	1.94	0.1726	2,55.3	0.50	0.6101
Gallery length (m)	1,61.14	16.88	0.0001	1,15.47	1.04	0.3243	1,34.1	1.40	0.2442
Log surface area (m^2)	1,46.82	0.07	0.7882	1,13.34	0.16	0.6935	1,36.5	0.62	0.4362
Mean tree phloem (mm)	1,26.35	0.11	0.7425	1,11.21	0.86	0.3734	1,11.9	0.85	0.3752
Sex	–	–	–	–	–	–	1,2817	134.34	<0.0001
Sex \times treatment	–	–	–	–	–	–	2,3012	3.01	0.0496
Sex \times Log height	–	–	–	–	–	–	1,2910	1.02	0.3135
Sex \times treatment \times Log height	–	–	–	–	–	–	2,3047	1.03	0.3577

Bold font indicates significance ($\alpha < 0.05$).

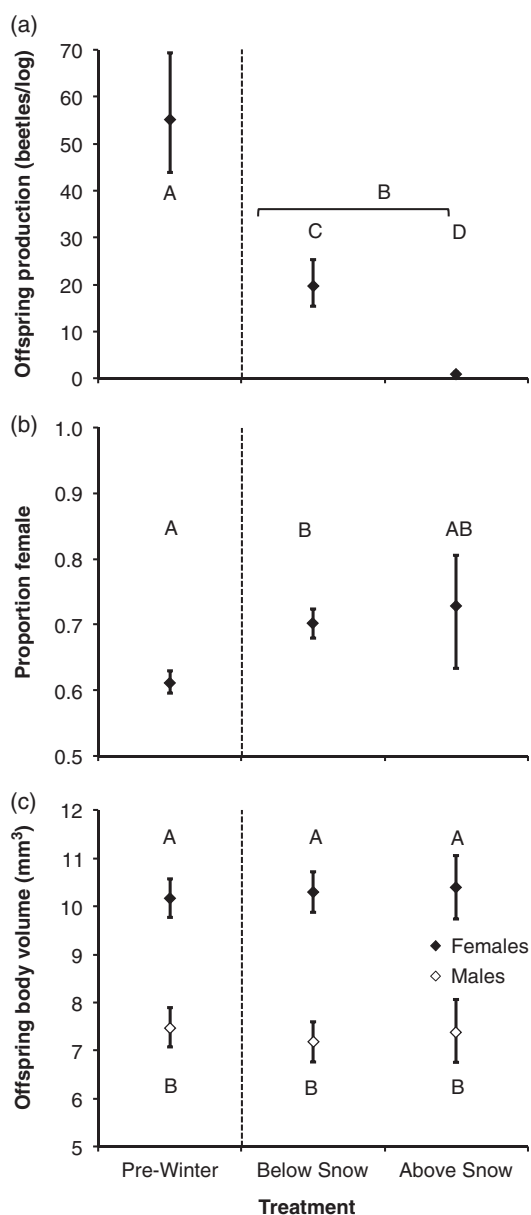


Fig. 3. The mean \pm (SE) (a) numbers of beetles produced per log, (b) sex ratio, and (c) body volume of female (closed symbols) and male (open symbols) beetles emerging from logs in each treatment. Based on back-transformed least square means from models reported in Table 2. Each treatment consisted of two logs from 15 trees. The vertical dashed line separates treatments that overwintered from the pre-winter treatment. Contrasting capital letters indicate significant differences. In (a) capital letters at the same height indicate significant difference based on *a priori* orthogonal contrasts.

gallery, beetles/m², and beetles per attack are summarised for each treatment (Table S1).

Mortality was also assessed for each log by estimating the number of beetles individual logs were expected to produce if they had not overwintered. The number of beetles that emerged from the pre-winter logs was explained primarily

by tree identity (overall $R^2 = 0.96$, $F_{17,11} = 16.57$, $P < 0.0001$; tree, $F_{14} = 11.03$, $P = 0.0002$; log height, $F_1 = 0.29$, $P = 0.60$; gallery length, $F_1 = 0.09$, $P = 0.77$; log surface area, $F_1 = 0.96$, $P = 0.35$). Using the model to determine the expected number of beetles, we created a mortality index (MI) as:

$$MI = 1 - [\log_e(P_{\text{observed}} + 1) / \log_e(P_{\text{expected}} + 1)],$$

where P_{observed} is the number of beetles produced per overwintered log from the same height (lower or upper) of the same tree for which a P_{expected} was estimated. A value of 0 indicates no overwintering mortality while a value of one indicates complete mortality; some slightly negative values occurred when more beetles were observed than expected. The mean MI (proportion of beetles that died) for below- and above-snow treatments were 0.32 ± 0.06 and 0.90 ± 0.03 , respectively ($n = 30$ for each treatment, $R^2 = 82.5$, $F_{2,69.2} = 118.32$, $P < 0.0001$).

Sex ratio

Overall, more females emerged from logs than males, but the sex ratio differed significantly among treatments. Pre-winter logs produced 61.2% female beetles (Fig. 3b), significantly greater than 50% (one sample t -test: $t_{25} = 6.58$, $P < 0.0001$). The sex ratios became even more female biased after overwintering (Fig. 3b and Table 2). Below-snow logs produced 70.2% female beetles, significantly more than observed for the pre-winter logs (post-hoc Tukey–Kramer test: $t_{31.54} = -3.56$, $Adj P = 0.0058$). The few beetles that emerged from above-snow logs were 72.8% female, but their small numbers (0.86 ± 0.55 beetles/log) prevented meaningful statistical comparisons of sex ratio. Sex ratio increased significantly with overwintering mortality index ($F_{1,61.17} = 4.94$, $P = 0.0299$, Fig. 4a).

Body volume

Body volume was marginally affected by overwintering. Prior to experiencing overwintering mortality the population displayed female-biased sexual size dimorphism as noted above (Fig. 3c). After statistically controlling for the length of maternal galleries, the surface area of the log, and phloem thickness, the effect of overwintering treatment on body volume differed significantly, albeit marginally, between males and females (Fig. 3c and Table 2; sex \times treatment interaction: $F_{2,3012} = 3.01$, $P = 0.0496$). The average body volume of females was greater than for males for each treatment. Although body volume was not detectably different among treatments within each sex, visual inspection of Fig. 3c suggests that female body volume was greater, and male body volume was smaller in the below-snow treatment than in the pre-winter treatment (sample size of the above-snow treatment was too small for meaningful comparisons). A similar trend was observed in the significant interaction between sex and mortality index for body volume (sex \times mortality index interaction: $F_{1,3047} = 3.85$, $P = 0.0498$; sex: $F_{1,3137} = 1254.99$, $P < 0.0001$; mortality index: $F_{1,98.9} = 0.02$, $P = 0.8892$). Although the effect was modest, as mortality increased per overwintered log, body volume of

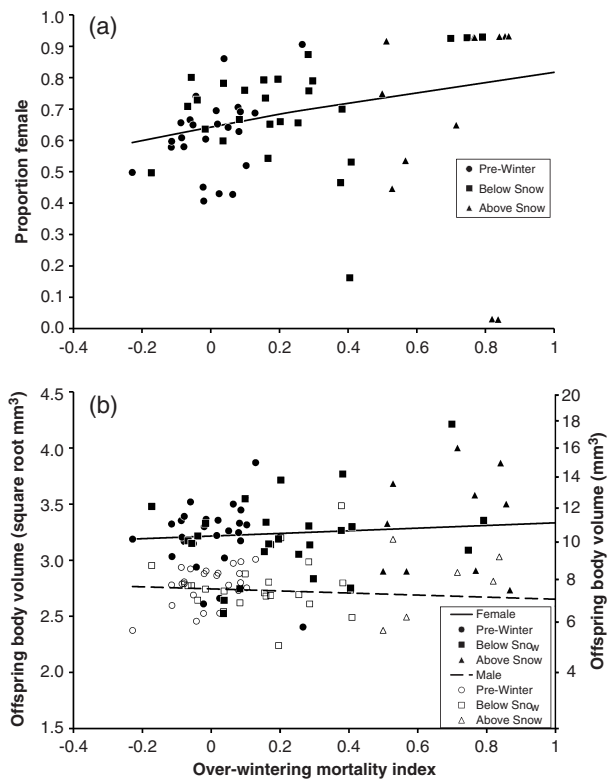


Fig. 4. The relations of (a) sex ratio and (b) offspring body volume of females (closed symbols, solid line) and males (open symbols, dashed line) to the overwintering mortality index. Data points are back-transformed estimates that account for the variation owing to random effects. Each data point represents the average body volume of all males or females that emerged from one log (two logs each from 15 trees). In (b), offspring body volume was square root transformed for analysis; the secondary axis presents the untransformed values.

emerging beetles tended to increase for females (coefficient: $0.1180 \pm 0.0970 \sqrt{\text{mm}^3}$), whereas it tended to decrease for males (coefficient: $-0.0916 \pm 0.1194 \sqrt{\text{mm}^3}$) (Fig. 4b).

Discussion

The major finding of this experimental field study was that male-biased mortality during overwintering contributes to female-biased adult sex ratios. Male mortality did not appear to be related to their relatively smaller body size. Male-biased mortality has implications for population dynamics of mountain pine beetles.

In this study, sex ratios of mountain pine beetles were set naturally before the start of the experiment, allowing us to detect changes in the sex ratio as a result of overwintering mortality. A female bias in the adult sex ratio existed prior to overwintering (61%), but became stronger in logs that overwintered (70–73%). Observed sex ratios were similar in magnitude to those reported in other studies in the laboratory without overwintering (64%: McGhehey, 1969; 62%: Safranyik, 1976; 57–75%: Amman & Bartos, 1991) and after natural emergence (56–69%: Safranyik

& Jahren, 1970; 58%: Gray *et al.*, 1972; 57–86%: Amman, 1984). In our study, sex ratios became more female-biased with greater mortality of juveniles, which indicates that males were not as successful at surviving winter. The higher mortality rate of males than females in the juvenile stage supports the sex-biased mortality previously observed in adults kept in cold storage (Safranyik, 1976). This is the first study, to our knowledge, that directly connects an increased female-biased sex ratio with juvenile mortality in mountain pine beetles.

If population sex ratios of mountain pine beetles were equal at oviposition, differential mortality of eggs and larvae may have shifted the sex ratio from equal at the time of laying to 61% female when pre-winter logs were moved to the laboratory on 4 December. Substantial mortality (70–95%) in broods before overwintering has been observed (Reid, 1963). In our study, overwinter mortality rates (64–98% of early winter brood) shifted the sex ratio a further 9–12% points in favour of females. Without knowing the primary sex ratio (of eggs), we cannot determine whether the initial bias is completely explained by mortality. Future studies will test whether a sex-ratio bias exists at the time of oviposition.

Male-biased mortality over winter skewed the adult sex ratio, and we hypothesised that smaller body size could make males more susceptible to the stress of overwintering temperatures or desiccation. Size-dependent mortality was expected to cause males to have a higher mortality rate than females because they are the smaller sex in mountain pine beetles (females were 37% larger in volume than males in our study). Size-dependent mortality has been demonstrated in the dragonfly *Libellula depressa* Linnaeus, in which smaller larvae suffered higher mortality independent of food availability (Wohlfahrt *et al.*, 2007). In the seed beetle *Callosobruchus maculatus* (Fabricius), differences in the degree of sexual size dimorphism at different rearing temperatures were suggested to result from size-selective mortality of males (Stillwell & Fox, 2007). Field studies of overwintering mountain pine beetles have found that cold exposure disproportionately killed early (smaller) instars relative to later (larger) instars (Amman, 1973; Safranyik *et al.*, 1974; Langor, 1989; Safranyik & Linton, 1998). However, laboratory measures of cold tolerance did not differ among instars (amount of glycerol: Sømme, 1964; and super cooling points: Bentz & Mullins, 1999), so vulnerability of early instars might instead be related to their smaller body size. We hypothesised that such size-dependent mortality would affect males disproportionately, assuming that they are smaller than females as juveniles as they are as adults. In contrast to our expectation that the smallest individuals of each sex would suffer the greatest mortality, leaving larger individuals to emerge, we saw little change in body size with mortality. To the extent that the sexes differed in the response of body size (marginal interactions between sex and treatment or mortality), the trend for body size to increase with mortality was evident only for females whereas body size decreased for males. Although the effects were weak, directional selection on female body size for survival could help maintain sexual dimorphism in this species in addition to the fecundity advantages of large female size (Honek, 1993).

An alternative explanation for increased body size of individuals that survived winter, compared with those from pre-winter

treatments, is that the high mortality could have reduced larval competition, facilitating growth once temperatures increased sufficiently for feeding to resume. Competition has been found to reduce body size in mountain pine beetles across maternal gallery densities comparable to ours (Amman & Pace, 1976). Relative differences in body size might matter more for contest than for scramble competition. Females might benefit more than males because their larger body size could favour them in contest competition (Anderson *et al.*, 1993) or if females are better able to acquire and use resources in general in scramble competition. Future work is merited to separate the effect of size-dependent over-wintering mortality and competition on final body size. Regardless, if reduced larval competition facilitated growth this should magnify any size-dependent mortality, so the weak changes in body size in both females and males further diminishes any support for the idea that smaller individuals were more likely to die.

Reasons for male-biased mortality, other than their smaller body size, remain unclear. It may be that cold tolerance depends more on lipid content than body size; the antifreeze agents such as glycerol may be generated from lipids, as suggested in other bark beetles (Lombardero *et al.*, 2000). In mountain pine beetles, pre-flight adult males have lower absolute and relative amounts of fat compared with females (Reid & Purcell, 2011; Graf *et al.*, 2012). Larval males may also have less fat and be unable to supercool as well as females, resulting in higher mortality. Rather than size, differences in fat stores or some other inherent difference in males may determine susceptibility to overwintering mortality.

Male mountain pine beetles may have higher mortality than females because they are the heterogametic sex (Lanier & Oliver, 1966). The heterogametic sex may suffer greater mortality as genes and deleterious mutations or recessive alleles on both sex chromosomes are exposed to selection (Crew, 1937; Myers, 1978). Greater mortality of developing female chicken embryos (ZW heterogametes) has been attributed to sex-linked lethal genes (Wu *et al.*, 2012). While greater mortality of the heterogametic sex could explain the sex ratio bias that exists even under benign conditions, such as in our pre-winter logs, it is unclear how this would be intensified in overwintering mountain pine beetles.

Male-killing cytoplasmic organisms (Hurst, 1991) such as *Wolbachia* are another potential mechanism causing the consistent female bias observed in mountain pine beetles. Another bark beetle with a female-biased sex ratio carries *Wolbachia* (Vega *et al.*, 2002) but *Wolbachia* has not been observed in mountain pine beetles (Bracewell *et al.*, 2010). Male-killing cytoplasmic factors usually cause early male death in their host, often at the embryo stage or during the first larval instar (Stouthamer *et al.*, 2002). Overwintering mortality affected the population after the egg stage, so cytoplasmic factors could contribute to the sex-ratio bias that exists in populations prior to overwintering but would not explain the increasing bias in sex ratio observed with greater overwinter mortality. Thus, further research is needed to understand why males are more likely to die over winter than are females.

Our study adds to the understanding of ecological consequences of winter conditions for mountain pine beetles. The

overwintering treatments significantly reduced the number of beetles that emerged, compared with the pre-winter logs. The insulating effect of snow (Stahl *et al.*, 2006) and reduced desiccation of the phloem (Cole, 1975) both likely contributed to the lower mortality in below-snow logs (64%) compared with above snow logs (98%). Only one other study has compared overwinter survival in relation to snow cover; mortality of overwintering adults was greater above snow (approximately 100% mortality) than below snow (approximately 50% mortality), but with no difference (approximately 2–15% mortality) for larvae (Reid, 1963). At that site, the minimum overwinter temperature of -25.6°C was milder than in ours (-33.7°C). At another site where temperatures reached -36.7°C , mortality of both larvae and adults was complete (Reid, 1963). Other studies of mountain pine beetle overwintering in lodgepole pine have reported similar rates of brood mortality under similar conditions (e.g. 95–100% mortality: Langor, 1989; 91–95%: Cole, 1981; 96.5%: Amman, 1984). A laboratory study of larvae in naturally infested logs showed mortality (22–80%) depended on larval instar, temperature, and duration of cold exposure (Safranyik & Linton, 1998). Early instars, colder temperatures, and longer durations caused greater mortality. The high mortality rates we observed may be because of larvae being predominantly early instar (Safranyik & Linton, 1998; but see Bentz & Mullins, 1999). Exposure to cold temperatures can decimate a mountain pine beetle population, but if there is deep snow accumulation a greater proportion of the colonised tree will be insulated against cold temperatures, thus enabling greater survival.

It is important to consider how environmental conditions might affect populations in the face of climate change. Cold winter temperatures are an important influence on mountain pine beetle population dynamics (Bolstad *et al.*, 1997; Carroll *et al.*, 2003; Stahl *et al.*, 2006). It is predicted for the Rocky Mountain region that winter temperatures will be warmer and precipitation more variable (Haughian *et al.*, 2012). If precipitation comes in the form of snow this may allow for greater survival of beetles over winter. However, if warmer temperatures cause a reduction in the maintenance of snow cover over winter, then beetles developing low on the bole may experience greater mortality and fluctuating overwinter temperatures will likely affect the beetles' ability to maintain their cold hardened state (Régnière & Bentz, 2007). Our study reveals that a disproportionate number of the surviving beetles will be female, the sex that initiates attacks on trees, which may allow for continued success at overcoming trees. Our results suggest that population modelling should account for both snow cover and sex ratio of survivors.

In summary, snow was found to be an important buffer against overwintering mortality. Female-biased sex ratios increased with overwinter mortality, consistent with previously suggested but untested predictions of male-biased mortality contributing to biased sex ratios. However, female-biased sex ratios existed in logs that did not overwinter, so additional mechanisms may be important for the sex ratio bias. The absence of a body size effect in males suggests that male-biased mortality was not size related. Further research is needed to understand mechanisms underlying the pervasive sex ratio bias in mountain pine beetles.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12108

Table S1. Summary of beetle productivity (mean \pm SE) for each treatment ($n = 30$).

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